Factors influencing *Prymnesium parvum* population dynamics during bloom initiation: Results from in-lake mesocosm experiments

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Running head:
P. parvum bloom formation

Abstract

The alga *Prymnesium parvum* forms large fish-killing blooms in many Texas (USA) lakes. In some of these lakes, however, *P. parvum* occurs but does not develop blooms. In this study, we investigated factors that may influence bloom initiation by conducting a series of in-lake experiments involving mixing of waters from L. Whitney, which has a history of *P. parvum* blooms, with waters from L. Waco where no blooms have occurred. In all experiments, the addition of L. Waco waters resulted in a poorer performance of *P. parvum*. Various experimental treatments and field data show that differences in grazing, pathogens, nutrients, and salts between the two lakes were not likely factors that contributed to this observation. Industrial and agricultural contaminants, allelochemicals and algicidal chemicals were not measured as a part of this research. However, anthropogenic contaminants other than nutrients were not observed at levels exceeding water quality standards in L. Waco in recent years. On the other hand, nuisance cyanobacteria are common in L. Waco, where *Microcystis* sp. and *Anabaena* sp. were abundant during the initiation of our experiments, both taxa are known to produce chemicals with allelopathic properties. In addition, the emergent field of algal-heterotrophic bacteria interactions suggests that chemicals produced by heterotrophic bacteria should not be overlooked. Further research focusing on the chemical interactions between cyanobacteria and *P. parvum*, as well as the potential role of algicidal bacteria, in the initiation of *P. parvum* blooms is necessary, as it may be important to the management of these blooms.

Keywords:
plankton, inorganic nutrients, salinity, grazing, harmful algal blooms, microcystins, prymnesins, allelopathy, cyanobacteria

Introduction

Prymnesium parvum is a haptophyte alga that occurs worldwide, tolerates large variations in temperature and salinity, and sometimes forms blooms that result in large fish kills (Edvardsen and Paasche 1998, Lundholm and Moestrup 2006, Baker et al. 2007). In Texas (USA), the incidence of *P. parvum* blooms has increased dramatically since 2001, where blooms are now observed in over 19 lakes found along five river basins. During blooms, surface waters take on a golden color with *P. parvum* cell densities typically exceeding 10×10^6 cells L^{-1}. In addition, fish kills occurring with these blooms involve many species, where total mortalities number in the tens of millions (TPWD 2003, Roelke et al. 2007).

Changes in the physicochemical environment that led to the increase in Texas *P. parvum* blooms are unknown, but might include eutrophication and salinization. For example, blooms in Europe, the Middle East, and Asia have all occurred in aquatic systems that were eutrophic and brackish (Krasnotshchek and Abramowitsch 1971, Holdway et al. 1978, Rijn and Shilo 1989, Kaartvedt et al. 1991, Guo et al. 1996, Amsinck et al. 2005). *P. parvum* blooms in Texas are partly consistent with these observations because they appear mostly in lakes that attain salinities 2 to 4 psu (practical salinity units) during low precipitation years (TPWD 2003), and these lakes may have experienced increased non-point source nutrient loading because of aging septic systems, point source discharges and expanded shoreline development. However, the role of nutrients is complex. In laboratory and field experiments the toxicity to fish from chemicals produced by *P. parvum*, which also act as allelochemicals and are an important factor leading to bloom initiation, was greater when cells were nutrient-limited (Uronen et al. 2005, Roelke et al. 2007, Errera et al. 2008). In addition, *P. parvum* is sensitive to pulses of nutrients where high

doses inhibited bloom formation (Barkoh et al. 2003, Grover et al. 2007, Kurten et al. 2007). Therefore, the temporal variation in nutrient availability is likely more important than a system's trophic state.

In general, factors leading to the formation of harmful algal blooms (HABs) are many and diverse (see Paerl 1988, Roelke and Buyukates 2001). In regards to *P. parvum*, bloom-initiating processes may include production of chemicals toxic to grazers (Granéli and Johansson 2003, Tillmann 2003, Barreiro et al. 2005, Calliari and Tiselius 2005, Roelke et al. 2007, Brooks et al. *Accepted, this issue*), use of alternative energy and nutrient sources through mixotrophy (Nygaard and Tobiesen 1993, Skovgaard and Hansen 2003), suppression of competitors through allelopathy (Fistarol et al. 2003, 2005, Granéli and Johansson 2003, Roelke et al. 2007, Errera et al. 2008, Grover et al. *Accepted, this issue*), and resistance to the allelopathic effects of other algae (Suikkanen et al. 2004, Tillmann et al. 2007). These factors are not likely mutually exclusive. Additional factors that seem to influence the growth of other HABs, which might also influence *P. parvum* population dynamics, include the production of beneficial or deleterious chemicals by various bacteria taxa (Kodama et al. 2006, Salomon and Imai 2006) and the pathogenic effects of some virus (Salomon and Imai 2006, Schwierzke et al. *In Review, this issue*).

Interestingly, *P. parvum* occurs in some Texas lakes where blooms do not form. At present, it is unclear why blooms do not develop in these lakes while occurring in neighboring reservoirs. As previously mentioned, factors influencing bloom formation might involve various aspects within the water environment ranging from zooplankton grazers to dissolved chemicals. The overall objective of this research was to determine the relative importance of these factors, or narrow down the number of potential influences on *P. parvum* bloom formation. To achieve this

goal, we conducted in-lake mesocosm experiments involving the mixing of waters from a lake frequented by *P. parvum* blooms with waters from a lake unaffected by blooms. The experimental treatments included manipulations of initial grazer and competitor population densities and community composition, along with manipulations of various ions and dissolved chemicals. Our experiments were conducted during the time of year when blooms start to develop in many Texas lakes.

Materials and Methods

L. Whitney is a reservoir on the Brazos River, Texas, USA, constructed in 1951. The lake receives drainage from an area of 42,107 km^2, has a capacity of 4.68×10^8 m^3, a surface area of 95 km^2, and a shoreline of 362 km (Bailes and Hudson 1982). River discharge in this developed watershed is flashy, with peak flows typically occurring in the late spring and early summer, lasting several days, which can result in hydraulic flushing peaks of ~0.25 d^{-1}. Discharge during the late summer, fall and winter months is low and sometimes undetectable. We chose an area further south in the lake for our study site (32°52.50, 97°23.10) where historically high *P. parvum* population densities have been observed during the late-fall through early-spring months, typically November through March (Glass, personal communication). L. Waco, a nearby reservoir from which waters were transported for these experiments, is located within the Bosque watershed. Relative to L. Whitney, L. Waco is smaller in area and capacity (78.7 km^2, 1.88×10^8 m^3) and drains less land area (4,325 km^2), but it is similar in geomorphology and hydrology, where river discharge in the late spring and early summer can result in hydraulic flushing peaks of ~0.33 d^{-1} (Dowell 1972, Lind and Barcena 2003).

We performed three in-lake experiments during a 5-week period in fall 2006, each lasting 7 days. Previous experiments conducted in a neighboring system observed significant phytoplankton and zooplankton responses to treatments within a 7-day period (Roelke et al. 2007, Errera et al. 2008). In experiments employing smaller volume containers, however, experimental artifacts were observed beyond the 7-day period (Errera et al. 2008). Consequently, we selected a 7-day duration for our experiments. Over short periods, the emerging plankton composition resulting from competition and other foodweb interactions can be sensitive to the initial community composition (Roelke et al. 2003, Roelke and Eldridge *In Press*). Since the community composition shifts during the time of year when blooms are forming in Texas lakes, possibly influencing the outcome of our treatments, we repeated three consecutive experiments, initiated October 3rd, 17th, and 31st.

In this study, we utilized 36 transparent 25-L polycarbonate carboys during each experiment, filled to a final volume of 24.5 L. Air in the headspace of each carboy allowed neutral buoyancy. The carboys were suspended in the near-surface water from anchored floatation platforms that allowed free movement with wave turbulence, keeping the carboys well mixed. To simulate the natural light environment, the carboys were covered with a neutral density screen, reducing surface light by ~55%. Lake secchi depths are typically ~1 m at this time of year, which translates to a light extinction coefficient of ~1.7 m^{-1} (see Wetzel 2001). A 55% reduction in surface light would then occur at ~0.5 m, the depth from which water was collected for experiment initiation. Measurements of pH taken at initiation and termination of the 7-day experiments suggested that CO_2 did not limit primary productivity in the sealed carboys.

Lake water used to initiate these experiments was collected from locations within L. Whitney and L. Waco away from the shoreline, where *P. parvum* occurred at the time. Profiles of temperature and salinity suggested that surface waters from both reservoirs were well mixed at the time of collection. Previous studies have identified that pH levels can influence the potency of prymnesins, toxins produced by *P. parvum* (Ulitzur and Shilo 1966, Shilo 1981). However, pH values between the lakes differed by only 0.25, with a mean value of 8.21. This slight variation did not cause differential potency effects between the lake waters.

Each experiment comprised 12 treatments that were conducted in triplicate. Five of the treatments represented a gradient of combined, unfiltered waters from L. Whitney and L. Waco at proportions of 100:0, 75:25, 50:50, 25:75 and 0:100 (Whitney:Waco). In these treatments, our goal was to assess the combined effect of grazers, competitors, pathogens and dissolved chemicals from L. Waco on population dynamics of *P. parvum*.

Another of our goals was to differentiate between the combined effects of grazers and competitors from L. Waco, from the combined effects of virus pathogens and dissolved chemicals from L. Waco on the population dynamics of *P. parvum.* To accomplish this, two additional treatments were performed. The first included a 50:50 mixed proportion of unfiltered L. Whitney water and 0.45 μm filtered (membrane filter) L. Waco water; the second, a 50:50 mixed proportion of unfiltered and 0.45 μm filtered L. Whitney waters. We assumed the filtered waters primarily contained dissolved constituents, viral particles, and bacteria <0.45 μm in cell size. Both treatments had the same initial population densities of phytoplankton and zooplankton, at 50% of natural abundances in L. Whitney, allowing us to account for potential effects of varied initial conditions on emergent community composition (Roelke et al. 2003, Roelke and Eldridge *In Press*).

Additional goals of our research were to investigate the roles of nutrients and salinity on *P. parvum* population dynamics when L. Waco and L. Whitney waters were mixed. To achieve this, a final five treatments were added. The first three involved mixed proportions of 100:0, 50:50 and 0:100 of unfiltered waters from L. Whitney and L. Waco with additions of inorganic nutrients, trace metals and vitamins to f/2 concentrations (Guillard and Ryther 1962). In regards to N and P, enrichment of N was to 800 μM-N and enrichment of P was to 40 μM-P (N:P ratio of 20). By enriching these treatments, the chance of growth limitation arising from the scarcity of inorganic nutrients, trace metals and vitamins was negated. The remaining two treatments included a 50:50 mixed proportion of waters with salts added, and a 50:50 mixed proportion of water from L. Whitney and 0.45 μm filtered water from L. Waco with salts added. Salinity levels were adjusted to match those found in L. Whitney. During our in-lake experiments, L. Waco salinity was ~0.14 psu, and L. Whitney salinity averaged 1.73 psu. A defined medium of artificial seawater (Kester 1967) was added to each carboy in order to reach the higher salinity characteristic of L. Whitney. Based on our observations while culturing the Texas strain of *P. parvum*, where inoculations are periodically performed into media with salinities varying by as much as 3 psu at low salinity ranges, it is not likely that an osmotic-shock due to a sudden shift of ~0.7 psu (from 1.73 to ~1 psu) influenced the outcome of our 7-day experiments.

Response variables measured in our experiments included characterizations of plankton, inorganic nutrients, and ambient toxicity (where toxicity refers to the net affect of all chemicals). Characterizations of plankton included estimates of total phytoplankton biomass and biomasses of higher taxonomic groups, enumerations of *P. parvum* population densities, and total zooplankton biomass and biomasses of higher taxonomic groups. Characterizations of the nutrients included measurements of nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), and

196 orthophosphate (PO_4). Ambient toxicity was estimated using standardized fish and cladoceran
197 bioassays previously described (Roelke et al. 2007). Initial conditions were characterized by
198 measurements taken just prior to the initiation of each experiment from both source waters.
199 Response variables were then sampled from each carboy at the end of each 7-day experiment.
200 Estimates of total phytoplankton biomass and biomasses of higher taxa were determined
201 from phytopigment concentration measurements (Pinckney et al. 1998) and the use of
202 CHEMTAX, a matrix factorization program that enables estimates of taxonomically aggregated
203 assemblage composition (Mackey et al. 1997, Wright et al. 1996). For the CHEMTAX model
204 initiation, cyanobacteria, euglenophytes, chlorophytes, prymnesiophytes, cryptophytes, diatoms
205 and chrysophytes were selected because of their historical prevalence in L. Whitney and L.
206 Waco. For greater detail of the HPLC and CHEMTAX methods followed, see Roelke et al.
207 (2007).
208 Water column chlorophyll *a* concentrations were also determined using standard
209 fluorometric procedures. Triplicate 50 mL samples were filtered through 47mm GF/F filters per
210 carboy and frozen until analysis. Pigments were extracted with 90% acetone, centrifuged, and
211 analyzed using a fluorometer (APHA 1998).
212 From each carboy a 100 mL phytoplankton sample was collected and preserved using
213 glutaraldehyde, 5% v/v. Enumeration of *P. parvum* population density was performed using a
214 settling technique (Utermöhl 1958). A subsample of 0.5 to 1.5 mL was settled for 24 h, then 30-
215 40 randomly selected fields of view were counted using an inverted, phase contrast light
216 microscope (400x, Leica Microsystems). Total cells counted per sample averaged between 50-
217 150.

Detailed microscopy was performed on L. Whitney and L. Waco waters used to initiate the experiments, following the same methods described above. The focus of this effort was to determine the proportion of the total phytoplankton biovolume that was represented by specific cyanobacteria taxa, i.e., genera-level characterizations that are not possible using the CHEMTAX model.

Zooplankton samples were collected following two methods. For initial in-lake conditions a Schindler trap (61 μm mesh size) was used to concentrate a 12 L sample to 50 ml. To sample carboys at the termination of each experiment, 10 L were removed and filtered through the cod end portion of a Schindler trap and concentrated to 50 ml. All zooplankton samples were preserved in buffered formalin, 5% v/v. A subsample of 5 to 15 mL was settled for 24 hours, then counted using an inverted, phase contrast light microscope (40x and 200x, Leica Microsystems). For each individual counted, dimensions were measured corresponding to best-fit geometric shapes to estimate biovolume (Wetzel and Likens 1991). For this study zooplankton species were grouped into total copepod adults, copepod nauplii, total rotifers, total cladocerans and total protozoans (ciliate and amoeboid). Our enumeration technique resulted in ~100-150 total individuals counted per sample.

Samples for inorganic nutrients were filtered through GF/F filters, and the filtrates were frozen until analysis. Inorganic nutrient concentrations were determined using autoanalyzer methodology (Armstrong and Sterns 1967, Harwood and Kuhn 1970). For this study, NO_3, NO_2 and NH_4 were summed (DIN).

Ambient acute toxicity to fish was evaluated for initial conditions and from each experimental carboy using standardized 24-hour static toxicity assays with the juvenile fathead minnow (*Pimephales promelas*) model. Sublethal toxicity to a model cladoceran was evaluated

241 with standardized 10-day static renewal chronic toxicity tests with the *Daphnia magna* model.
242 Toxicity assays followed standardized methods for aquatic toxicology (US EPA 1994, 2002).
243 Samples were collected and transported to the laboratory where toxicity tests were initiated
244 within 24 hours. Ambient samples were diluted using a 0.5 dilution series with reconstituted
245 hard water (RHW), which was performed according to US EPA recommendations (US EPA
246 2002). For greater detail of the methods followed for these toxicity assays, refer to Brooks et al.
247 (2004) and Dzialowski et al. (2006).

248 Using SPSS 14, comparisons of the response variables ([day 7 – day 0]/day 0) between
249 experimental treatments involving the five mixed proportions of unfiltered waters from L.
250 Whitney and L. Waco were tested using one-way ANOVA, followed by Bonferroni and Tukey
251 *post hoc* tests. Independent samples t-tests were performed for all other treatment comparisons.
252 These comparisons focused on the differences of primary interest in this study: effects of the
253 source and proportion of waters mixed in the experimental carboys.
254
255 **Results**
256 *In-situ conditions for L. Whitney and L. Waco*

257 From the three sampling dates in L. Whitney (October 3rd, 17th and 31st), *P. parvum* cell
258 densities were 0.72 x 10^6, 0.50 x 10^6, and 0.83 x 10^6 cells L^{-1}, well below bloom proportions in
259 Texas waters (~10 x 10^6 cells L^{-1}), and toxicity bioassays confirmed non-toxic in-lake conditions.
260 The state fisheries managers indicated no observations of golden-colored waters and no reports of
261 fish kills at this time (Glass, personal communication). Foam lines were present, however, which
262 typically correspond with the incidence of *P. parvum*. Approximately one month after our in-
263 lake experiments golden-colored waters and fish kills were observed. Therefore, we conclude that

these experiments took place during the period of bloom initiation.

Cyanobacteria dominated the phytoplankton assemblage (50% of the biovolume, almost all *Phormidium* sp.; Table 1) during the October 3rd sampling of L. Whitney. During the later two October samplings, phytoplankton composition was more diverse, with co-dominance of chlorophytes, chrysophytes, cyanobacteria and prymnesiophytes, and sub-dominance of euglenophytes and cryptophytes. Diatoms were below detection based on CHEMTAX analysis of pigments, and cyanobacteria known to be harmful were not observed in microscopy (Table 1). Total phytoplankton biomass fluctuated during the three lake samplings, with concentrations of 17.9, 25.9 and 19.8 μg-chl *a* L^{-1}.

Total zooplankton biovolume was similar for the first two samplings in L. Whitney, but then increased ~10-fold over the study period. On October 3rd and 17th biovolumes were 3.0 x 10^7 and 1.3 x 10^7 μm^3 L^{-1}, but on October 31st total biovolume was 13.8 x 10^7 μm^3 L^{-1}. Rotifers accounted for 86% of the total biovolume on October 3rd (101 individuals L^{-1}), but by October 17th rotifers declined to 37% (16 individuals L^{-1}) and copepod nauplii comprised 63% (10 individuals L^{-1}) of the total biovolume. By October 31st rotifers became subdominant (15%, 70 individuals L^{-1}), and copepod nauplii (44%, 103 individuals L^{-1}) and copepod adults (34%, 7 individuals L^{-1}) were co-dominant. Cladocerans, and ciliated and amoeboid protozoa were not observed.

L. Waco was sampled on the same dates as L. Whitney, and *P. parvum* cell densities were very similar at 0.31 x 10^6, 0.37 x 10^6, and 0.62 x 10^6 cells L^{-1}. Consistent with this lake's history, there were no signs of *P. parvum* blooms during the period of our experiments. To date, there have still been no observed *P. parvum* blooms in L. Waco.

286 During October, total phytoplankton biomass in L. Waco was similar to levels found in L.
287 Whitney: 17.4, 19.6 and 21.5 μg-chl *a* L^{-1} for the three sampling dates in L. Waco. However,
288 cyanobacteria were much more prevalent compared to L. Whitney, accounting for 77%, 69% and
289 51% of the total phytoplankton biovolume for the three sampling dates. In addition, potentially
290 toxigenic species were observed in our microscopy, where *Microcystis* sp. and *Anabaena* sp.
291 were dominant (Table 1).

292 Zooplankton varied in L. Waco during October, with total biovolumes of 10.7 x 10^7, 1.3 x
293 10^7 and 10.0 x 10^7 μm^3 L^{-1} for the three sampling dates. Similar to L. Whitney, initial rotifer
294 dominance gave way to co-dominance with copepod nauplii. On October 3rd rotifers accounted
295 for 98% (114 individuals L^{-1}) of the total zooplankton biovolume, but on October 17th and 31st,
296 rotifers accounted for 41% and 53% of the total biovolume (25 and 93 individuals L^{-1}). Copepod
297 nauplii shared dominance at 57% and 37% of the total biovolume (11 and 46 individuals L^{-1})
298 during the latter two sampling dates. Similar to L. Whitney, cladoceran and ciliated and amoeboid
299 protozoa population densities were very low in L. Waco during this time.

300 Inorganic nutrient concentrations were similar between the two lakes. In L. Whitney,
301 average DIN and PO_4 concentrations (from the three lake samples collected at the start of each
302 experiment) were 3.8 μM-N and 0.10 μM-P, and in L. Waco they were 2.78 μM-N and 0.22
303 μM-P. The ratio of N:P varied, however; L. Whitney N:P was ~38, while the L. Waco N:P was
304 ~13.

305 *Experimental results*

306 The accumulation of *P. parvum* populations in the experimental carboys varied according
307 to the proportions of waters mixed from L. Whitney and L. Waco. In all three experiments, *P.*

parvum increased 3- to 7-fold in carboys with only unfiltered L. Whitney water, while appreciable increases were not observed in the carboys with only unfiltered L. Waco water (Fig. 1). Furthermore, a dose-response was observed in all three experiments where the accumulation of *P. parvum* populations diminished with an increasing proportion of unfiltered L. Waco water. Distinct groupings along this mixing gradient were statistically significant ($p < 0.05$). Low accumulation of *P. parvum* was also observed in the carboys with filtered waters from L. Waco in all three experiments (Fig. 1). Statistically significant reductions in fish survival and cladoceran reproduction responses were never observed, including in the carboys with greater proportions of L. Whitney water where *P. parvum* population densities reached near-bloom proportions.

For phytoplankton biomass, mixing of unfiltered waters from L. Whitney and L. Waco did not produce a consistent trend as seen with *P. parvum* (Fig. 2). Reductions in chlorophyll *a* from initial conditions occurred, and the decrease was greater in carboys containing larger proportions of L. Whitney water in the first two experiments, but the opposite was observed for the third experiment.

The phytoplankton response in the carboys containing filtered L. Waco waters was consistent with the trends observed for *P. parvum*, where in all three experiments performance of the total phytoplankton assemblage was poorer in carboys with L. Waco waters, with significant differences ($p < 0.05$) for the first and third experiments (Fig. 2). Not all taxa were affected similarly. For example, cyanobacteria, euglenophytes and cryptophytes showed significantly ($p < 0.05$) poorer performance with the addition of filtered water from L. Waco. A poorer performance of chlorophytes was also observed, but only significant ($p < 0.05$) in the third experiment. Chrysophytes showed no significant difference between these treatments (Table 2).

330 Diatoms remained below the detection threshold for pigment analysis with the CHEMTAX
331 model.

332 Zooplankton biovolume increased from initial conditions in all carboys. In the first two
333 experiments the growth of zooplankton biovolume was significantly greater in carboys with a
334 larger proportion of L. Waco waters ($p < 0.05$, Fig. 3). Growth of copepod nauplii into adult
335 forms was pronounced in the second and third experiments. The accumulation of zooplankton
336 biovolume in the treatments involving filtered waters from L. Waco was not significantly
337 different from the treatments involving filtered waters from L. Whitney ($p > 0.05$, Fig. 3).

338 The addition of nutrients showed varying results. Enrichment did not change the
339 deleterious effect of filtered L. Waco waters on *P. parvum* populations ($p < 0.05$, Fig. 4). On the
340 other hand, nutrient additions changed the phytoplankton response to addition of filtered L.
341 Waco waters, but the effect was not consistent between experiments. During the first and third
342 experiments, there was no significant difference in phytoplankton growth between enriched
343 carboys with filtered waters from L. Whitney and L. Waco. During the second experiment,
344 however, phytoplankton growth was significantly greater in the enriched carboys with filtered L.
345 Whitney water. Shifts in assemblage composition between the higher taxonomic groups were
346 minor, except in carboys with 50% filtered L. Whitney waters where euglenophytes dropped
347 below the detection level of pigment analysis based on the CHEMTAX model. Accumulation of
348 zooplankton biovolume was much greater with nutrient additions, primarily due to the
349 population growth of rotifers. Differences between filtered water from L. Whitney and from L.
350 Waco, however, were not observed in any of the three experiments.

351 Salt additions to mixtures of L. Whitney and L. Waco waters, bringing salinity levels up

352 to those observed in L. Whitney (from ~1 to 1.7 psu), did not alter results in any experiment.
353 That is, no significant differences ($p < 0.05$) were found between L. Whitney and L. Waco waters
354 (50:50) without salt added compared to salt additions for *P. parvum*, chlorophyll *a*, or
355 zooplankton biovolume (Fig. 5). Similarly, no significant differences ($p < 0.05$) were found
356 between L. Whitney and filtered L. Waco waters (50:50) without salt added compared to salt
357 additions for these same parameters (Fig. 6). During the first experiment, notable increases in
358 rotifers were observed with the salt additions, but they were highly variable between replicates.
359 L. Whitney and L. Waco are similar in their trophic state, and this resulted in comparable
360 nutrient concentrations among various treatments. Excluding the carboys with enrichment,
361 nutrients ranged from 0.9 to 1.1 μM-DIN and 0.13 to 0.15 μM-PO_4 at the termination of all three
362 experiments.
363
364 **Discussion**
365 Waters from L. Waco, when mixed with waters from L. Whitney, had a deleterious impact
366 on *P. parvum* in these experiments. Zooplankton prospered in all treatments, with rapid growth
367 of parthenogenetic rotifers and the development of copepod nauplii into adults. Undoubtedly,
368 grazing pressure increased during the 7 days of each experiment. It was not the zooplankton
369 from L. Waco, however, that led to the poorer performance of *P. parvum* because both unfiltered
370 and filtered waters from L. Waco produced the same deleterious response. In addition,
371 accumulation of zooplankton biomass differed significantly between mixing treatments only in
372 the first experiment, where copepod adults increased in biomass. Remaining potential causes for
373 poor performance of *P. parvum* in L. Waco waters then, were some small pathogen passing

374 through a 0.45 μm filter, possibly a virus, or dissolved chemicals, such as a nutrients, salts,
375 contaminants, allelochemicals or algicides.

376 The role of pathogens in phytoplankton population dynamics is becoming clearer with
377 advances in technologies enhancing study in this area. Viruses directly influence other harmful
378 algal species (Brussaard 2004, Salomon and Imai 2006), and there is evidence that viruses
379 influence *P. parvum* population dynamics during later stages of blooms (Schwierzke et al. *In*
380 *Review, this issue*). However, pathogens tend to be species-specific and do not affect the entire
381 phytoplankton assemblage (Tomaru et al. 2004, Salomon and Imai 2006). In all three of our
382 experiments, additions of filtered L. Waco waters resulted in poorer performance of
383 cyanobacteria, euglenophytes and cryptophytes, which led to an overall poorer performance of
384 the entire L. Whitney phytoplankton assemblage (based on chlorophyll *a*). In addition, signs of
385 pathogens, such as lysed cells, were not observed during microscopy. These lines of evidence
386 indicate that the causative factor in L. Waco waters reducing performance of *P. parvum* was not
387 likely a pathogen.

388 A more generalized factor that could influence *P. parvum* and the phytoplankton
389 assemblage would be nutrients. However, based on half-saturation coefficients for reproductive
390 growth of many phytoplankton species common to lakes (Grover 1989, Grover et al. 1999),
391 nutrient concentrations would not have been a growth-limiting factor for many species, especially
392 at the start of these experiments. For a Texas strain of *P. parvum*, half-saturation constants for
393 N- and P-limited reproductive growth were estimated to be 0.02 μM or lower (Baker 2007, Baker
394 et al. *Accepted*), indicating that reproductive growth rates would be near maximum during these
395 experiments. More conclusively, the same deleterious effect of filtered L. Waco water on *P.*

396 *parvum* was observed in carboys with nutrient enrichment. Further, ammonium levels did not
397 approach those toxic to *P. parvum* or other algae in this study and did not vary appreciably
398 among treatments. For these reasons, it is not likely that differences in nutrient concentrations
399 between L. Whitney and L. Waco led to the poorer performance of *P. parvum*.

400 For bulk phytoplankton, nutrient limitation would be expected to begin when
401 concentrations drop below 1.0 μM-N and 0.1 μM-P (Reynolds 2006). Interestingly, in the first
402 and third experiments nutrient additions masked the deleterious effect of L. Waco waters when
403 considering the whole phytoplankton community (based on chlorophyll *a*). While major shifts in
404 the aggregated taxonomic groups were not observed, it may be that species shifts within each of
405 the aggregated groups occurred. For example, species resistant to the deleterious effect of L.
406 Waco waters, which could have higher nutrient requirements, might have prospered with the
407 addition of nutrients while sensitive species declined.

408 Another generalized factor that could influence *P. parvum* and the phytoplankton
409 assemblage is salinity. For *P. parvum*, relationships between growth rate and salinity are
410 unimodal or increasing, depending on the strain (Larsen and Bryant 1998). A strain of *P. parvum*
411 from Texas showed a unimodal relationship with an optimum at 22 psu (Baker et al. 2007). The
412 salinity at the time of our experiments was much lower than that, differing by ~1.3 psu between
413 the two reservoirs, and a 50:50 mix of these waters yielded a salinity of ~1 psu. While this
414 salinity is low, it is sufficient to support growth of *P. parvum* when other conditions are
415 favorable (Baker 2007, Baker et al., *Accepted*).

416 Applying the relationship between growth and salinity described by Baker (2007), the
417 specific growth rates of *P. parvum* would have been ~0.3 and ~0.15 d^{-1} under conditions of ~1.7

418 and ~1 psu. In the treatments where filtered L. Waco waters were mixed with L. Whitney waters
419 and salts were not added (i.e., salinity was not maintained at 1.73), the difference in salinity could
420 have contributed to the differences observed in *P. parvum* population densities. However, when
421 salinity was maintained with the addition of filtered L. Waco waters, the same deleterious effect
422 of filtered L. Waco waters on *P. parvum* was observed, as it was for total phytoplankton.
423 Therefore, the difference in salt content between the two lakes did not likely cause the negative
424 impact of L. Waco waters on *P. parvum* or the phytoplankton assemblage. Our conclusion is not
425 that salinity has no influence on the incidence of *P. parvum* blooms, as observations suggest
426 otherwise (Krasnotshchek and Abramowitsch 1971, Holdway et al. 1978, Rijn and Shilo 1989,
427 Kaartvedt et al. 1991, Guo et al. 1996, Amsinck et al. 2005). Instead, this indicates that another
428 factor, aside from salinity, played a more significant role in these experiments.
429 Industrial and agricultural contaminants would also act as generalized factors potentially
430 influencing the entire phytoplankton assemblage. The recent history of L. Waco shows a wide
431 range of contaminants being present, but of the many parameters routinely measured by the
432 Texas Commission on Environmental Quality, only NO_3 was of concern. Parameters not of
433 concern included a suite of heavy metals, polychlorinated biphenyls, organic contaminants
434 (including herbicides), fecal coliform bacteria, suspended sediments, NH_4 and PO_4. Overall, the
435 lake was found to be fully supporting of aquatic life, including phytoplankton (TCEQ 2004).
436 While total phytoplankton is not threatened by present contaminants, individual species might
437 be. Species-specific affects of contaminants have not been studied for L. Waco or L. Whitney,
438 and are well beyond the scope of this research. However, a negative effect on *P. parvum* and
439 major components of the phytoplankton assemblage (cyanobacteria, euglenophytes and

440 cryptophytes) from contaminants in L. Waco during these experiments is unlikely. On-going
441 monitoring in L. Waco shows accumulation of phytoplankton biomass with diverse taxa and a
442 sustained, but generally small, *P. parvum* population (Roelke, unpublished). If contaminants had
443 an affect similar to the responses measured in our experiments, cyanobacteria, euglenophytes,
444 cryptophytes and *P. parvum* populations would occur at much lower densities then currently
445 observed in L. Waco.

446 Allelopathy is another generalized factor potentially affecting *P. parvum* and other
447 phytoplankton. *P. parvum* is capable of producing chemicals that suppress the growth of
448 competing phytoplankton (Fistarol et al. 2003, 2005, Granéli and Johansson 2003, Roelke et al.
449 2007, Errera et al. 2008), as can other harmful species, including *Microcystis* sp. and *Anabaena*
450 sp. (Pflugmacher 2002, Legrand et al. 2003). During our experiments, both of these latter taxa
451 were abundant in waters collected from L. Waco (possibly due to the relatively low N:P) and
452 were absent in waters collected from L. Whitney. Furthermore, in 2006, microcystin-LR was
453 detected by ELISA at levels between 590 and 1090 ng L^{-1} in L. Waco (Brooks, unpublished),
454 although production of this toxin is not necessary to cause an allelopathic affect (see Sukenik et
455 al. 2002, Beresovsky et al. 2006). Dissolved allelochemicals would have passed through filtration
456 and been present in treatments receiving filtered L. Waco water. Thus, allelopathy could explain
457 why performances of major taxonomic groups in L. Whitney declined with the addition of L.
458 Waco water.

459 How might *P. parvum* respond to allelochemicals from cyanobacteria? Previous research
460 using a European strain of *P. parvum* indicated a resistance to allelochemicals in studies
461 performed at 6.8 psu (Suikkanen et al. 2004). However, the Texas and European strains have

462 different photopigments (Errera 2006) and may also vary in vulnerabilities to allelochemicals.
463 The possibility of compounding chemical interactions exists as well. The higher ambient pH of
464 Texas lakes (Roelke, unpublished), relative to the Baltic Sea, may influence the lipophilicity,
465 bioavailability and toxicity of allelochemicals. So, it may be that *P. parvum* populations in L.
466 Whitney are sensitive to cyanobacterial allelochemicals. In addition, a modeling study of L.
467 Granbury, another Texas lake impacted by *P. parvum* blooms, has demonstrated that model
468 behavior better matched in-lake observations of *P. parvum* blooms when allelochemicals from
469 cyanobacteria were considered (Grover et al., *Accepted, this issue*). In addition to cyanobacteria,
470 allelopathy is observed in many other phytoplankton taxa (Granéli and Hansen 2006), therefore
471 allelochemicals from other phytoplankton species in L. Waco cannot be ruled out.
472 Algicidal chemicals are produced by diverse bacteria and may be another generalized
473 factor potentially affecting *P. parvum* and other phytoplankton (Kodama et al. 2006, Salomon
474 and Imai 2006). The impacts of algicides are also varied. Some broadly affect many
475 phytoplankton taxa, while others are species-specific; in some cases phytoplankton growth is
476 slowed, and in others target populations are obliterated (Doucette et al. 1998, Imai et al. 1998,
477 Yoshinaga et al. 1998, Mayali and Azam 2004). Elucidating the possible role algicidal bacteria
478 played in these experiments was well beyond the scope of this study, but future research should
479 address this possible bloom-suppressing mechanism.
480 Allelochemicals produced by cyanobacteria, some other phytoplankton taxa, or algicidal
481 chemicals produced by bacteria may be, in part, contributing factors leading to the suppression of
482 *P. parvum* blooms in L. Waco, and perhaps in some other regional lakes as well. For example, in
483 L. Somerville, *P. parvum* is present but does not form blooms. Similar to L. Waco, this lake is

484 frequently dominated by cyanobacteria (Roelke et al. 2004), and microcystins were detected. In
485 addition, nutrients, dissolved organic carbon, and bacteria concentrations are high. The potential
486 roles of allelochemical-producing phytoplankton and algicidal bacteria as ecosystem-engineering
487 species or in suppressing *P. parvum* blooms in Texas lakes merits further investigation.

488 As stated previously, the environmental conditions leading to harmful algal blooms are
489 complex and often species-specific, making it difficult to envision a universal approach to
490 management (Roelke 2000, Roelke and Buyukates 2001, 2002). Our findings, along with those of
491 Grover et al. (*Accepted, this issue*), suggest that allelopathic phytoplankton or algicidal bacteria
492 might influence *P. parvum* bloom development. Should follow-on studies confirm this notion,
493 then our findings would have important management implications for *P. parvum* in Texas lakes.
494 For example, a management strategy could focus on the understanding of conditions where *P.*
495 *parvum* blooms do not occur, e.g., in the presence of some allelopathic phytoplankton or algicidal
496 bacteria. Manipulating an entire lake to create conditions conducive to a specific microbe may
497 not be feasible, or even wise. However, manipulation of more restricted areas of a lake, timed
498 during the season of bloom initiation, would likely be less detrimental to the overall health of a
499 lake. Targeting specific coves of the dendritic lakes common in this region would be ideal.
500 Ongoing research is investigating the role of coves as bloom initiation "hot spots", possibly due
501 to their longer hydrologic residence time. If true, then a focused, timely effort to promote growth
502 of specific allelopathic phytoplankton or algicidal bacteria, if these are confirmed to suppress *P.*
503 *parvum*, in these coves might help to circumvent *P. parvum* blooms. Even if blooms initiate
504 elsewhere, manipulation in coves may still be advantageous to ward off *P. parvum* blooms,
505 thereby creating a refuge for fish.

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References

American Public Health Association, American Water Works Association, Water Environment Foundation, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC, USA.

Amsinck S.L., E. Jeppesen, F. Landkildehus, 2005. Inference of past changes in zooplankton community structure and planktivorous fish abundance from sedimentary subfossils - a study of a coastal lake subjected to major fish kill incidents during the past century. Archiv für Hydrobiol. 162:363-382.

Armstrong, F.A., C.R. Sterns, 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. Deep-Sea Res I 14:381-389.

Bailes, C., Hudson, D.L, 1982. A Guide to Texas Lakes, including the Brazos, Colorado, Frio, and Guadalupe Rivers. Houston: Pacesetter Press.

Baker, J.W., 2007. Basic ecology and mathematical modeling of *Prymnesium parvum*, "golden algae", in Texas. Ph.D. Dissertation, University of Texas at Arlington.

528 Baker, J.W., J.P. Grover, B.W. Brooks, F. Ureña-Boeck, D.L. Roelke, R.M. Errera, R. Kiesling,
529 2007. Growth and toxicity of *Prymnesium parvum* (Haptophyta) as a function of salinity,
530 light and temperature. J. Phycol. 43:219-227.
531 Baker J.W., J.P. Grover, R. Ramachandrannair, C. Black, T.W. Valenti, Jr., B.W. Brooks, D.L.
532 Roelke. (*Accepted*) Dynamics at the edge of the niche: an experimental study of the harmful
533 alga *Prymnesium parvum*. Limnology and Oceanography.
534 Barkoh, A., D.G. Smith, J.W. Schlechte, 2003. An effective minimum concentration of un-
535 ionized ammonia nitrogen for controlling *Prymnesium parvum*. N. Am. J. Aquacult. 65: 220-
536 225.
537 Barreiro A., C. Guisande, I. Maneiro, T.P. Lien, C. Legrand, T. Tamminen, S. Lehtinen, P.
538 Uronen, E. Granéli E, 2005. Relative importance of the different negative effects of the toxic
539 haptophyte *Prymnesium parvum* on *Rhodomonas salina* and *Brachionus plicatilis.* Aquat.
540 Microb. Ecol. 38:259-267.
541 Beresovsky D., O. Hadas A. Livne, A. Sukenik, A. Kaplan, S. Carmelia, 2006. Toxins and
542 biologically active secondary metabolites of *Microcystis* sp. isolated from Lake Kinneret.
543 Israel J. Chem. 46:79-87.
544 Brooks, B.W., J.K. Stanley, J.C. White, P.K. Turner, K.B. Wu, T.W. La Point, 2004.
545 Laboratory and field responses to cadmium in effluent-dominated stream mesocosms.
546 Environ. Tox. Chem. 24:464-469.
547 Brooks, B.W., S.V. James, T.W. Valenti Jr., F. Urena-Boeck, C. Serrano, L. Schwierzke, L.D.
548 Mydlarz, J.P. Grover, D.L. Roelke. (*Accepted, this issue*). Comparative toxicity of
549 *Prymnesium parvum* in inland waters. Journal of American Water Resources Association.

550 Brussaard, C.P.D., 2004. Viral control of phytoplankton populati – a review. J. Eukary.
551 Microbiol. 51:125-138.
552 Calliari D., P. Tiselius, 2005. Feeding and reproduction in a small calanoid copepod: *Acartia*
553 *clausi* can compensate quality with quantity. Mar. Ecol. Prog. Ser. 298:241-250.
554 Doucette, G.J., M. Kodama, S. Franca, S. Gallacher, 1998. Bacterial interactions with harmful
555 algal bloom species: bloom ecology, toxigenesis, and cytology. In Anderson, D.M., A.D.
556 Cembella, G.M. Hallegraeff (eds) Physiological Ecology of Harmful Algal Blooms, Springer,
557 New York, pp. 619-647.
558 Dowell, C.L. 1972. Dams and Reservoirs in Texas: History and Descriptive Information, Texas
559 Water Commission Bulletin 6408, Dayton Kelley (ed.). The Handbook of Waco and
560 McLennan County, Texas.
561 Dzialowski E.M., P.K. Turner, B.W. Brooks, 2006. Physiological and reproductive effects of β-
562 adrenergic receptor antagonists on *Daphnia magna*. Archiv. Environ. Contam. Tox. 50:503-
563 510.
564 Edvardsen B, E. Paasche, 1998. Bloom dynamics and physiology of *Prymnesium* and
565 *Chrysochromulina*. pp. 193-208. In Anderson DM, Cembella AD, Hallegraff GM [eds.]
566 The physiological ecology of harmful algal blooms. Springer-Verlag, Heidelberg.
567 Errera, R.M., 2006. Inhibition and success of *Prymnesium parvum* invasion on plankton
568 communities in Texas, USA and *Prymnesium parvum* pigment dynamics. M.S. thesis,
569 Department of Wildlife and Fisheries Sciences, Texas A&M University, 169 p.
570 Errera, R.M., D. Roelke, R. Kiesling, B.W. Brooks, J.P. Grover, L. Schwierzke, F. Ureña-Boeck,
571 J.W. Baker, J.L. Pinckney, 2008. The effect of imbalanced nutrients and immigration on

572 *Prymnesium parvum* community dominance and toxicity: Results from in-lake microcosm
573 experiments, Texas, USA. Aquat. Microb. Ecol. 52: 33-44.
574 Fistarol G.O , C. Legrand, E. Granéli, 2003. Allelopathic effect of *Prymnesium parvum* on a
575 natural plankton community. Mar. Ecol. Prog. Ser. 255:115-125.
576 Fistarol G.O., C. Legrand, E. Granéli, 2005. Allelopathic effect on a nutrient-limited
577 phytoplankton species. Aquat. Microb. Ecol. 41:153-161.
578 Granéli E., N. Johansson, 2003. Effects of the toxic haptophyte *Prymnesium parvum* on the
579 survival and feeding of a ciliate: the influence of different nutrient conditions. Mar. Ecol.
580 Prog. Ser. 254:49-56.
581 Granéli E., P.J. Hansen, 2006. Allelopathy in harmful algae: A mechanism to compete for
582 resources? pp. 189-201. *In* E. Graneli and J.T. Turner (Eds.) Ecology of Harmful Algae.
583 Springer-Verlag, Berlin.
584 Grover, J.P., 1989. Phosphorus-dependent growth kinetics of 11 species of freshwater algae.
585 Limnol. Oceanogr. 34, 341-348.
586 Grover, J.P., R.W. Sterner, J.L. Robinson, 1999. Algal growth in warm temperate reservoirs:
587 nutrient-dependent kinetics of individual taxa and seasonal patterns of dominance. Archiv für
588 Hydrobiol. 145:1-23.
589 Grover, J.P., J.W. Baker, F. Ureña-Boeck, B.W. Brooks, R. Errera, D.L. Roelke, R.L. Kiesling,
590 2007. Laboratory tests of ammonium and barley straw extract as agents to suppress
591 abundance of the harmful alga Prymnesium parvum and its toxicity to fish. Water Res. 41:
592 2503-2512.
593 Grover J.P., J.W. Baker, D.L. Roelke, B,W. Brooks. (*Accepted*, this issue) Mathematical models

of population dynamics of *Prymnesium parvum* in inland waters. Journal of American Water Resources Association.

Guillard, R.R.L., J.H. Ryther, 1962. Studies of marine plankton diatoms. Can. J. Microbiol. 8:229-239.

Guo M., P.J. Harrison, F.J.R. Taylor, 1996. Fish kills related to *Prymnesium parvum* N. Carter (Haptophyta) in the Peoples Republic of China. J. Appl. Phycol. 8:111-117.

Harwood, J.E., A.L. Kuhn, 1970. A colorimetric method for ammonia in natural waters. Water Res. 4:805-811.

Holdway P.A., R.A. Watson, B. Moss, 1978. Aspects of the ecology of *Prymnesium parvum* (Haptophyta) and water chemistry in Norfolk Broads, England. Freshwater Biol. 8:295-311.

Imai, I., M.C. Kim, K. Nagasaki, S. Itakura, Y. Ishida, 1998. Relationships between dynamics of red ride-causing raphidophycean flagellates and algicidal micro-organisms in the coastal sea of Japan. Phycol. Res. 46:139-146.

Kaartvedt S., T.M. Johnsen, D.L. Aksnes, U. Lie, 1991. Occurrence of the toxic phytoplagellate *Prymnesium parvum* and associated fish mortality in a Norwegian fjord system. Can. J. Fish. Aquat. Sci. 48:2316-2323.

Kester, D.R., Duedell, I.W., Connors, D.N., Pytkowicz, R.M., 1967. Preparation of artificial seawater. Limnol. Oceanogr. 12:176-9.

Kodama, M., G.J. Doucette, D.H. Green, 2006. Relationships between bacteria and harmful algae. pp. 243-258. *In* E. Graneli and J.T. Turner (Eds.) Ecology of Harmful Algae. Springer-Verlag, Berlin.

Krasnotshchek G.P., L.S. Abramowitsch, 1971. Mass development of *Prymnesium parvum*

Cart. in fish breeding ponds. Hydrobiol. 7:54-55.

Kurten, G.L., A. Barkoh, L.T. Fries, D.C. Begley, 2007. Combined nitrogen and phosphorus fertilization for controlling the toxigenic alga *Prymnesium parvum*. N. Am. J. Aquacult. 69: 214-222.

Larsen, A., S. Bryant, 1998. Growth rate and toxicity of *Prymnesium parvum* and *Prymnesium patelliferum* (Haptophyta) in response to changes in salinity, light and temperature. Sarsia. 83: 409-418.

Legrand, C, K. Rengefors, G.O. Fistarol, E. Graneli, 2003. Allelopathy in phytoplankton - biochemical, ecological and evolutionary aspects. Phycologia. 42: 406-419.

Lind, O.T., E. Barcena, 2003. Response of riverine and transition zone bacterioplankton communities to a pulsed river inflow. Hydrobiol. 504: 79-85.

Lundholm, N., O. Moestrup, 2006. The biogeography of harmful algae. pp. 23-35. *In* E. Graneli and J.T. Turner [Eds.], Ecology of Harmful Algae. Springer-Verlag, Berlin.

Mackey, M., D. Mackey, H. Higgins, S. Wright, 1997. CHEMTAX—a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar. Ecol. Prog. Ser. 144: 265-83.

Mayali X., F. Azam, 2004. Algicidal bacteria in the sea and their impact on algal blooms. J. Eukaryot. Microbiol. 51:139-144.

Nygaard K., A. Tobiesen, 1993. Bacterivory in algae – A survival strategy during nutrient limitation. Limnol. Oceanogr. 39:273-279.

Paerl, H.W., 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol. Oceanogr. 33:823-847.

638 Pinckney J.L., H.W. Paerl, M.B. Harrington, K.E. Howe, 1998. Annual cycles of
639 phytoplankton community structure and bloom dynamics in the Neuse River Estuary, North
640 Carolina. Mar. Biol. 131:371-82.
641 Pflugmacher, S., 2002. Possible allelopathic effects of cyanotoxins, with reference to
642 microcystin-LR, in aquatic ecosystems. Environ. Tox. 17: 407-413.
643 Reynolds, C.S., 2006. Ecology of Phytoplankton. Cambridge University Press, Cambridge. 535
644 p.
645 Rijn van J, M. Shilo., 1989. Environmental factors in fish culture systems. pp. 163-177. *In*
646 Shilo M, Sarig S [eds], Fish culture in warm water systems: Problems and Trends, CRC
647 Press, Inc., Boca Raton, Florida.
648 Roelke D.L., 2000. Copepod Food-Quality Threshold as a Mechanism Influencing
649 Phytoplankton Succession and Accumulation of Biomass, and Secondary Productivity: A
650 Modeling Study with Management Implications. Ecol. Model. 134:245-274.
651 Roelke D.L., Y. Buyukates, 2001. The Diversity of Harmful Algal Bloom-Triggering
652 Mechanisms and the Complexity of Bloom Initiation. Hum. Ecol. Risk Assess. 7:1347-1362.
653 Roelke D.L., Y. Buyukates, 2002. Dynamics of phytoplankton succession coupled to species
654 diversity as a system-level tool for study of *Microcystis* population dynamics in eutrophic
655 lakes. Limnol. Oceanogr. 47: 1109-1118.
656 Roelke, D.L., S. Augustine, and Y. Buyukates. 2003. Fundamental predictability in multispecies
657 competition: The influence of large disturbance. Am. Nat. 162: 615-623.
658 Roelke, D.L., Y. Buyukates, M. Williams, and J. Jean, 2004. Interannual variability in the
659 seasonal plankton succession of a shallow, warm-water lake. Hydrobiol.. 513: 205-218.

660 Roelke D.L., R. Errera, R. Kiesling, B.W. Brooks, J.P. Grover, L. Schwierzke, F. Ureña-Boeck, J.
661 Baker, J.L. Pinckney, 2007. Effects of nutrient enrichment on *Prymnesium parvum*
662 population dynamics and toxicity: Results from field experiments, Lake Possum Kingdom,
663 USA. Aquat. Microb. Ecol. 46:125-140.
664 Roelke D.L., Eldridge P.M. Losers in the 'Rock-Paper-Scissors' game: The role of non-
665 hierarchical competition and chaos as biodiversity sustaining agents in aquatic systems.
666 Ecological Modelling. *In Press*.
667 Salomon, P.S. and I. Imai, 2006. Pathogens of Harmful Microalgae. pp. 271-282. *In* E. Graneli
668 and J.T. Turner (Eds.) Ecology of Harmful Algae. Springer-Verlag, Berlin.
669 Schwierzke, L., D.L. Roelke, B.W. Brooks, J.P. Grover, T.W. Valenti, Jr., M. Lahousse, C.J.
670 Miller, J.L. Pinckney. (*In Review, this issue*) The role of grazers and viruses in *Prymnesium*
671 *parvum* bloom development: In situ experiments from a subtropical lake. Journal of
672 American Water Resources Association.
673 Shilo, M., 1981. The Toxic Principles of *Prymnesium parvum*. pp. 37-47. In W. W. Carmichael
674 (Ed.) The Water Environment: Algal Toxins and Health. Plenum Press, N.Y.
675 Skovgaard A., P.J. Hansen, 2003. Food uptake in the harmful alga *Prymnesium parvum*
676 mediated by excreted toxins. Limnol. Oceanogr. 48:1161-1166.
677 Sommer, U., Z. M. Gliwicz, W. Lampert and A. Duncan, 1986. The PEG-model of seasonal
678 succession of plankton events in fresh waters. Archiv für Hydrobiol. 106:436-440.
679 SPSS, Incorporated. Professional Statistics v.14. Chicago, Illinois.
680 Suikkanen S., G.O. Fistarol, E. Granéli, 2004. Allelopathic effects of the Baltic cyanobacteria
681 *Nodularia spumigena, Aphanizomenon flos-aquae* and *Anabaena lemmermannii* on algal

682 monocultures. J. Exp. Mar. Biol. Ecol. 308:85-101.

683 Sukenik, A., R. Eshkol, A. Livne, O. Hadas, M. Rom, D. Tchernov, A. Vardi, A. Kaplan, 2002.

684 Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by

685 *Microcystis* sp (cyanobacteria): A novel allelopathic mechanism. Limnol. Oceanogr. 47:1656-

686 1663.

687 TCEQ, 2004. Guidance for Assessing Texas Surface and Finished Drinking Water Quality Data.

688 Texas Commission on Environmental Quality, Office of Compliance and Enforcement and

689 Monitoring Operations Division, Surface Water Quality Monitoring Program. Brazos River

690 Basin, Lake Waco: 2004 Assessment. 9 pp.

691 TDSHS, 2006. Characterization of Potential Adverse Health Effects of Consuming Fish from

692 Waco Lake, McLennan County, TX. Texas Department of State Health Services, Austin,

693 TX. 30 pp.

694 TPWD, 2003. *Prymnesium parvum* Workshop Report. Texas Parks & Wildlife Department,

695 Austin, TX. (http://www.tpwd.state.tx.us/landwater/water/environconcerns/hab/).

696 Tillmann, U., 2003. Kill and eat your predator: a winning strategy of the planktonic flagellate

697 *Prymnesium parvum*. Aquat. Microbial. Ecol. 32:73-84.

698 Tillmann, U., U. John, A. Cembella, 2007. On the allelochemical potency of the marine

699 dinoflagellate *Alexandrium ostenfeldii* against heterotrophic and autotrophic protists. J. Plank.

700 Res. 29:527-543.

701 Tomaru, Y., N. Katanozaka, K. Nishida, Y. Shirai, K. Tarutani, M. Yamaguchi, K. Nagasaki,

702 2004. Isolation and characterization of two distinct types of HcRNAV, a single-stranded

703 RNA virus infecting the bivalve-killing microalga *Heterocapsa circularisquama*. Aquat.

704 Microb. Ecol. 34:207-218.

705 Ulitzer, S., M. Shilo, 1966. Mode of action of *Prymnesium parvum* ichthyotoxin. J. Protozool.

706 13: 332-336.

707 U.S. Environmental Protection Agency, 1994. 10-day Chronic Toxicity Test Using *Daphnia*

708 *magna* or *Daphnia pulex*. EPA SOP#2028. Environmental Response Team, United States

709 Environmental Protection Agency, Washington, DC.

710 U.S. Environmental Protection Agency, 2002. Methods for measuring the acute toxicity of

711 effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012.

712 United States Environmental Protection Agency, Washington, DC.

713 Uronen P., S. Lehtinen, C. Legrand, P. Kuuppo, T. Tamminen, 2005. Haemolytic activity and

714 allelopathy of the haptophyte *Prymnesium parvum* in nutrient-limited and balanced growth

715 conditions. Mar. Ecol. Prog. Ser. 299:137-148.

716 Utermöhl, H., 1958. Zur Vervollkominnung der quantitativen phytoplankton methodik. Mitt.

717 Int. Ver. Theoret. Ang. Limnol. 9:1-38.

718 Wetzel, R.G., G.E. Likens, 1991. Limnological Analysis. Springer-Verlag, New York.

719 Wetzel, R.G., 2001. Limnology, 3rd ed. Acedemic Press, New York.

720 Wright S., D. Thomas, H. Marchant, H. Higgins, M. Mackey, D. Mackey, 1996. Analysis of

721 phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy

722 and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX'

723 matrix factorization program. Mar. Ecol. Prog. Ser. 144:285-298.

724 Yoshinaga, I., M.C. Kim, N. Katanozaka, I. Imai, A. Uchida, Y. Ishida, 1998. Population

725 structure of algicidal marine bacteria targeting the red tide forming alga *Heterosigma akashiwo*

726 (Raphidophyceae), determined by restriction fragmental length polymorphism analysis of the
727 bacterial 16S ribosomal RNA genes. Mar. Ecol. Prog. Ser. 170:33-44.
728

728 **Figure Captions**

729

730 Fig. 1. Population density of *P. parvum* at the start (filled squares) and termination (bars) after 7
731 days of mixing experiments initiated on October 3rd (A), 17th (B) and 31st (C). Waters
732 represented a gradient of mixing proportions between unfiltered waters from L. Whitney and L.
733 Waco; 50% unfiltered L. Whitney waters added to 50% filtered L. Whitney waters; and 50%
734 unfiltered L. Whitney waters added to 50% filtered L. Waco waters. Letter designations
735 represent distinct groupings ($p < 0.05$).

736

737 Fig. 2. Phytoplankton biomass, as estimated by chlorophyll *a* concentration, at the start (filled
738 squares) and termination (bars) after 7 days of mixing experiments initiated on October 3rd (A),
739 17th (B) and 31st (C). Waters represented a gradient of mixing proportions between unfiltered
740 waters from L. Whitney and L. Waco; 50% unfiltered L. Whitney waters added to 50% filtered
741 L. Whitney waters; and 50% unfiltered L. Whitney waters added to 50% filtered L. Waco waters.
742 Letter designations represent distinct groupings ($p < 0.05$).

743

744 Fig. 3. Average zooplankton biovolume and community composition at the start (filled squares)
745 and termination (stacked bars) after 7 days of mixing experiments initiated on October 3rd (A),
746 17th (B) and 31st (C). Waters represented a gradient of mixing proportions between unfiltered
747 waters from L. Whitney and L. Waco; 50% unfiltered L. Whitney waters added to 50% filtered
748 L. Whitney waters; and 50% unfiltered L. Whitney waters added to 50% filtered L. Waco waters.
749 Letter designations represent distinct groupings ($p < 0.05$). No letter designations signify no
750 significant differences were detected..

751

752 Fig. 4. Impact of L. Waco waters with the addition of nutrients to population density of *P.*
753 *parvum,* phytoplankton biomass, and average zooplankton biovolume and composition at the
754 start (filled squares) and termination (bars) after 7 days for experiments initiated on October 3rd
755 (A), 17th (B) and 31st (C), where the treatments were 50% unfiltered L. Whitney waters added to
756 50% filtered L. Whitney waters; and 50% unfiltered L. Whitney waters added to 50% filtered L.
757 Waco waters. In all experiments, L. Waco waters had a deleterious effect on *P. parvum*
758 population growth ($p < 0.5$). Phytoplankton response was varied, however, where opposite
759 trends in the first two experiments (A, B) were significant ($p < 0.05$), and not significant in the
760 third experiment (C). Filtered L. Waco waters had no detectable impact on zooplankton.

761

762 Fig. 5. Impact of L. Waco waters with the addition of salts to match L. Whitney salinities to
763 population density of *P. parvum,* phytoplankton biomass, and average zooplankton biovolume
764 and composition at the start (filled squares) and termination (bars) after 7 days for experiments
765 initiated on October 3rd (A), 17th (B) and 31st (C), where the treatments were 50% unfiltered L.
766 Waco waters added to 50% L. Whitney waters; and 50% unfiltered L. Waco waters added to
767 50% L. Whitney waters with salt added. In all experiments, no significant difference ($p < 0.5$)
768 was detected for *P. parvum* population growth, or accumulation of phytoplankton and
769 zooplankton biomass.

770

771 Fig. 6. Impact of filtered L. Waco waters with the addition of salts to match L. Whitney
772 salinities to population density of *P. parvum,* phytoplankton biomass, and average zooplankton
773 biovolume and composition at the start (filled squares) and termination (bars) after 7 days for

774 experiments initiated on October 3rd (A), 17th (B) and 31st (C), where the treatments were 50%
775 filtered L. Waco waters added to 50% L. Whitney waters; and 50% filtered L. Waco waters
776 added to 50% L. Whitney waters with salt added. In all experiments, no significant difference (p
777 < 0.5) was detected for *P. parvum* population growth, or accumulation of phytoplankton and
778 zooplankton biomass.

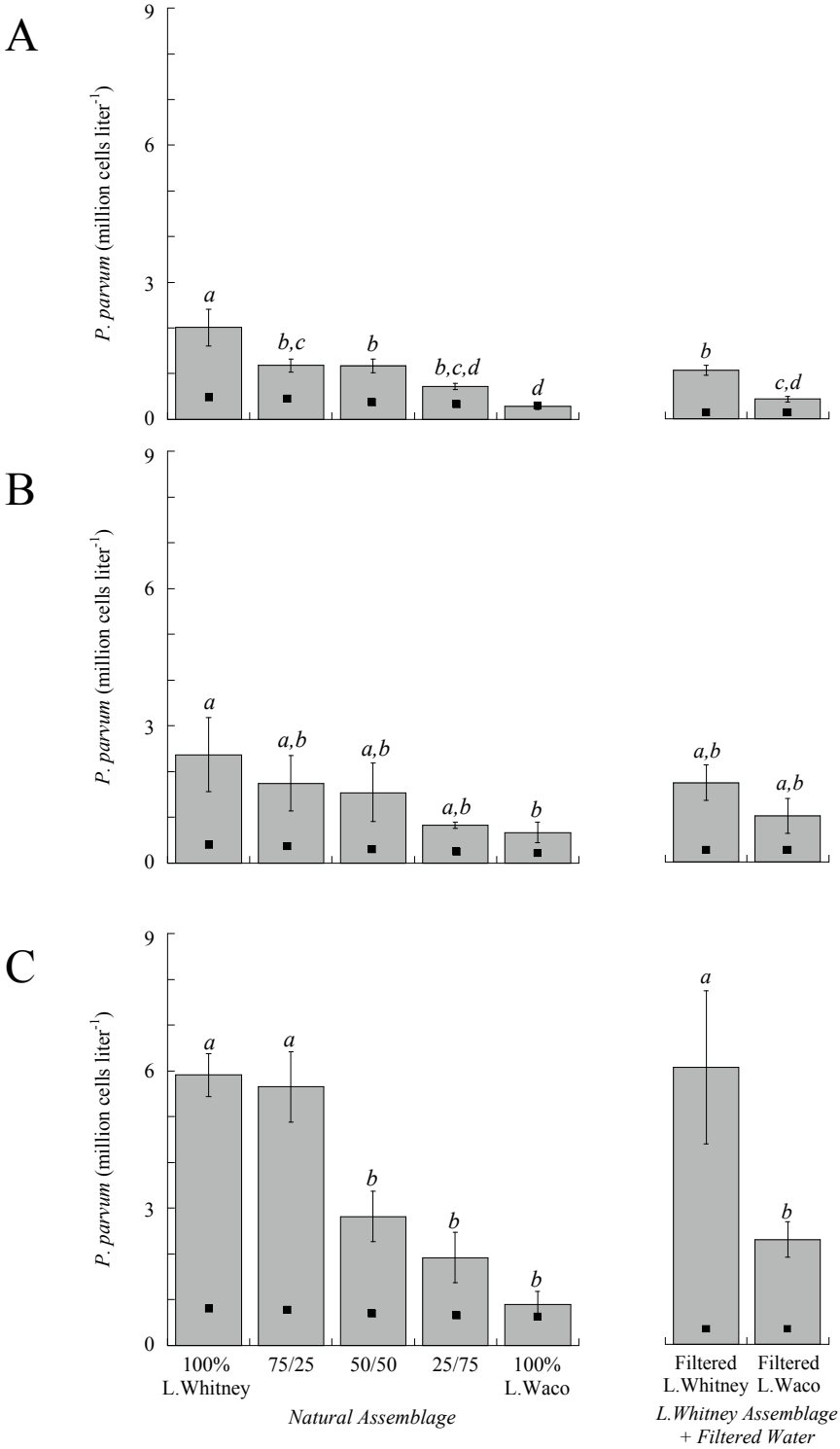
A
B
C
P. parvum (million cells liter-1)
9
6
3
0
a
b,c
b
b,c,d
d
b
c,d
a
a,b
a,b
a,b
b
a,b
a,b
a
a
b
b
b
b
100% L.Whitney
75/25
50/50
25/75
100% L.Waco
Natural Assemblage
Filtered L.Whitney
Filtered L.Waco
L.Whitney Assemblage + Filtered Water

Fig. 1

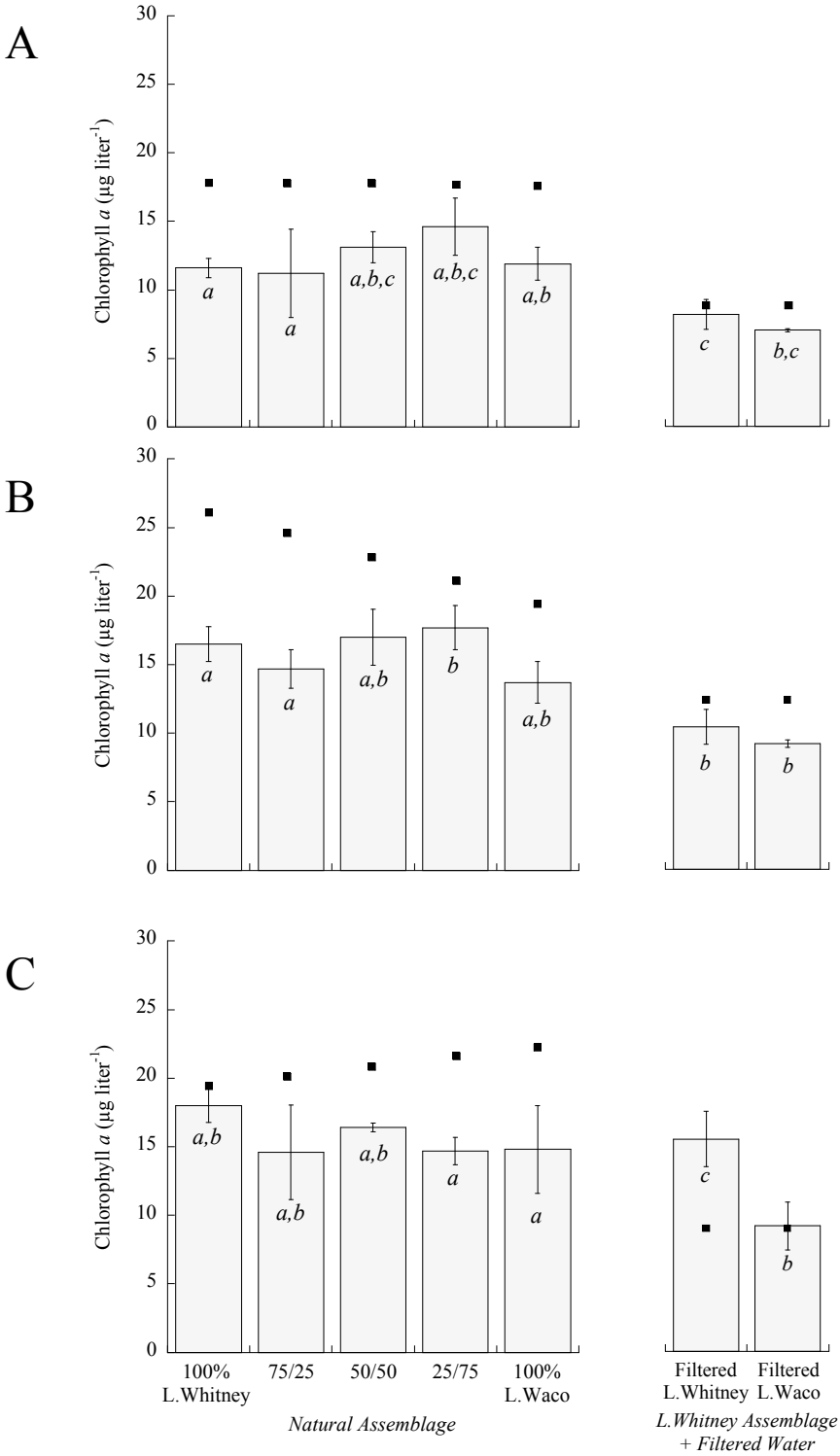
A
B
C
Chlorophyll a (µg liter-1)
30
25
20
15
10
5
0
a
a
a,b,c
a,b,c
a,b
c
b,c
a
a
a,b
b
a,b
b
b
a,b
a,b
a,b
a
a
c
b
100% L.Whitney
75/25
50/50
25/75
100% L.Waco
Natural Assemblage
Filtered L.Whitney
Filtered L.Waco
L.Whitney Assemblage + Filtered Water

Fig. 2

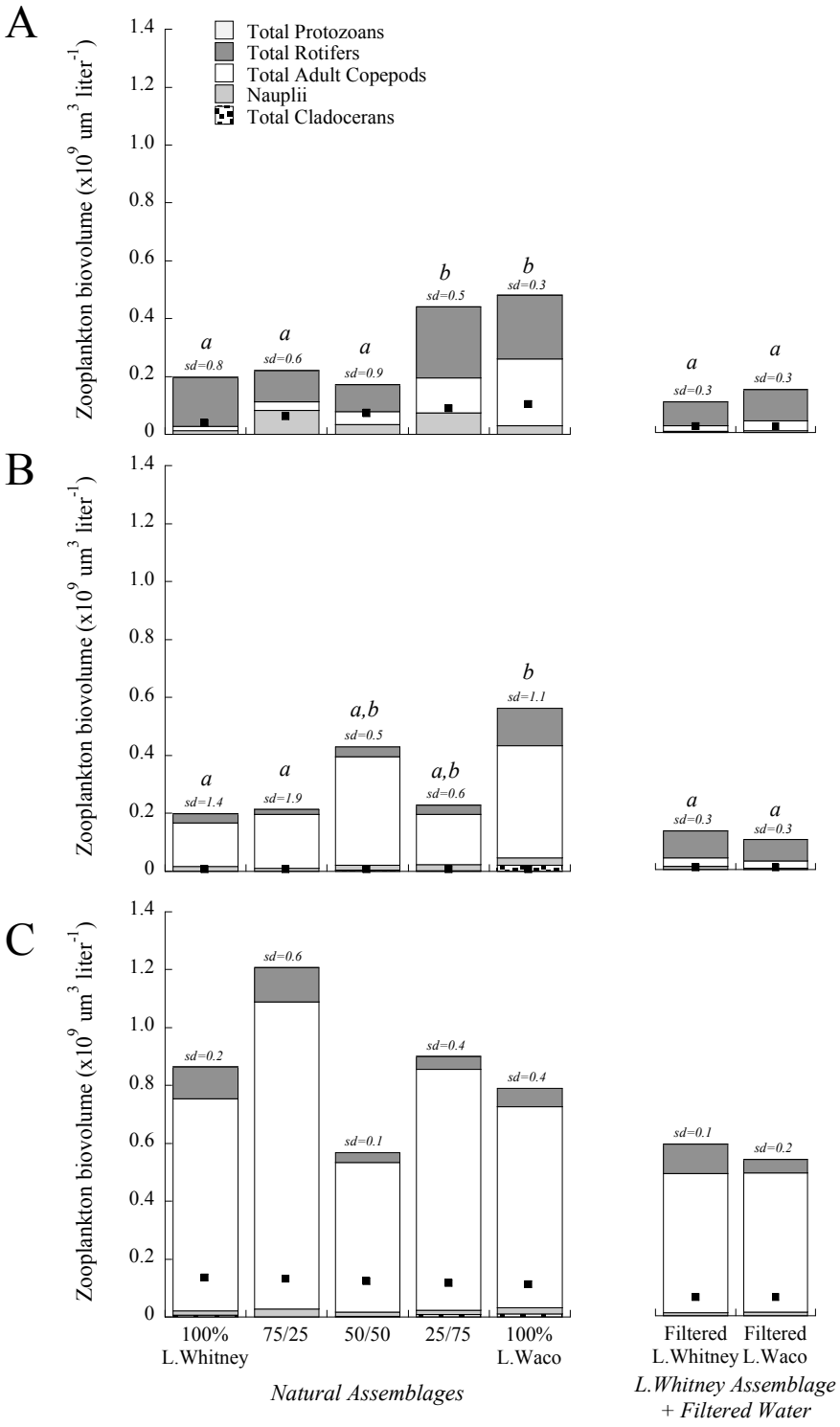
A
B
C
Total Protozoans
Total Rotifers
Total Adult Copepods
Nauplii
Total Cladocerans
Zooplankton biovolume (x10^9 um^3 liter^-1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
a
sd=0.8
a
sd=0.6
a
sd=0.9
b
sd=0.5
b
sd=0.3
a
sd=0.3
a
sd=0.3
a
sd=1.4
a
sd=1.9
a,b
sd=0.5
a,b
sd=0.6
b
sd=1.1
a
sd=0.3
a
sd=0.3
sd=0.2
sd=0.6
sd=0.1
sd=0.4
sd=0.4
sd=0.1
sd=0.2
100% L.Whitney
75/25
50/50
25/75
100% L.Waco
Filtered L.Whitney
Filtered L.Waco
Natural Assemblages
L.Whitney Assemblage + Filtered Water

Fig. 3

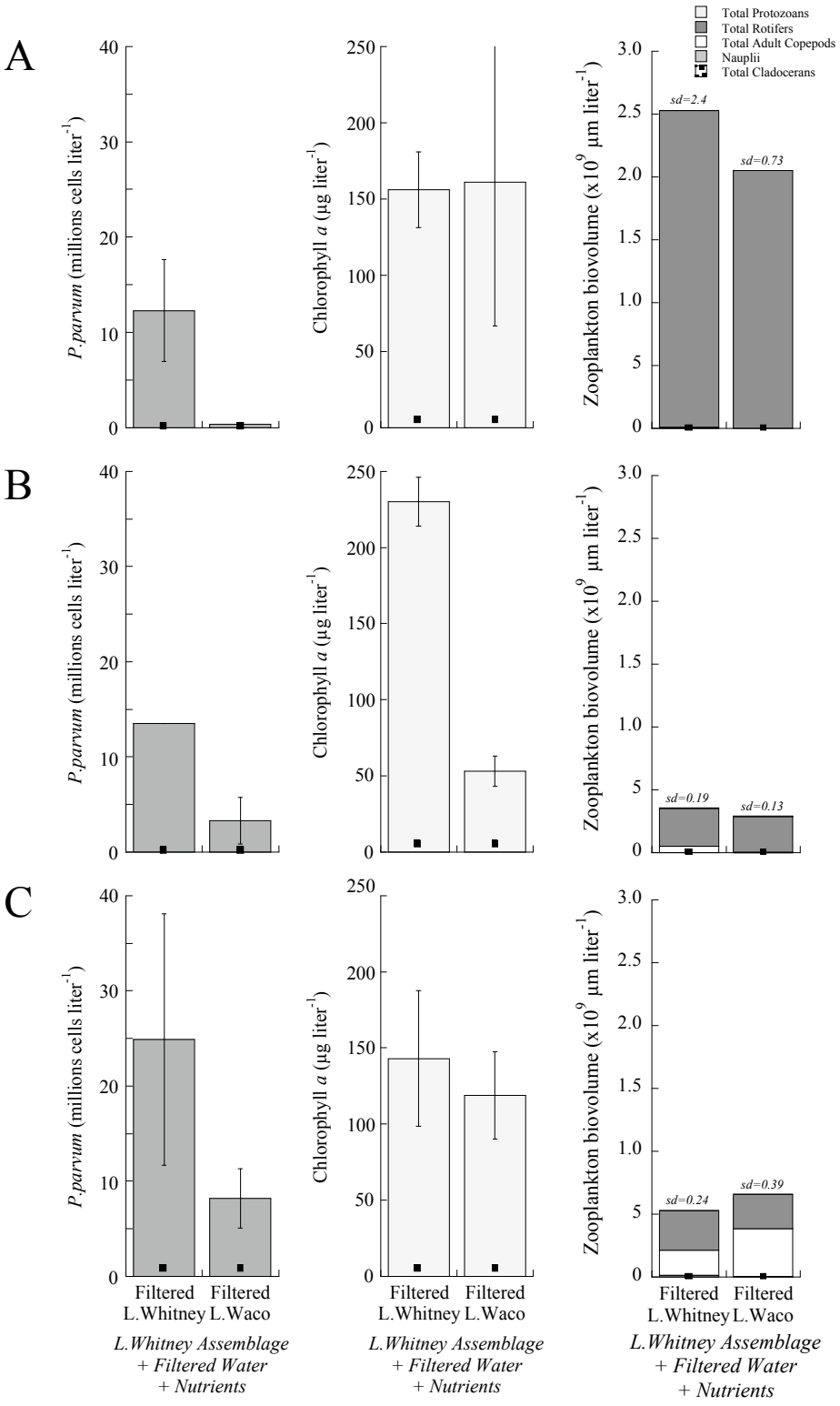
A
B
C
Total Protozoans
Total Rotifers
Total Adult Copepods
Nauplii
Total Cladocerans
P.parvum (millions cells liter-1)
Chlorophyll a (µg liter-1)
Zooplankton biovolume (x10^9 µm liter-1)
sd=2.4
sd=0.73
sd=0.19
sd=0.13
sd=0.24
sd=0.39
Filtered L.Whitney
Filtered L.Waco
L.Whitney Assemblage + Filtered Water + Nutrients

Fig. 4

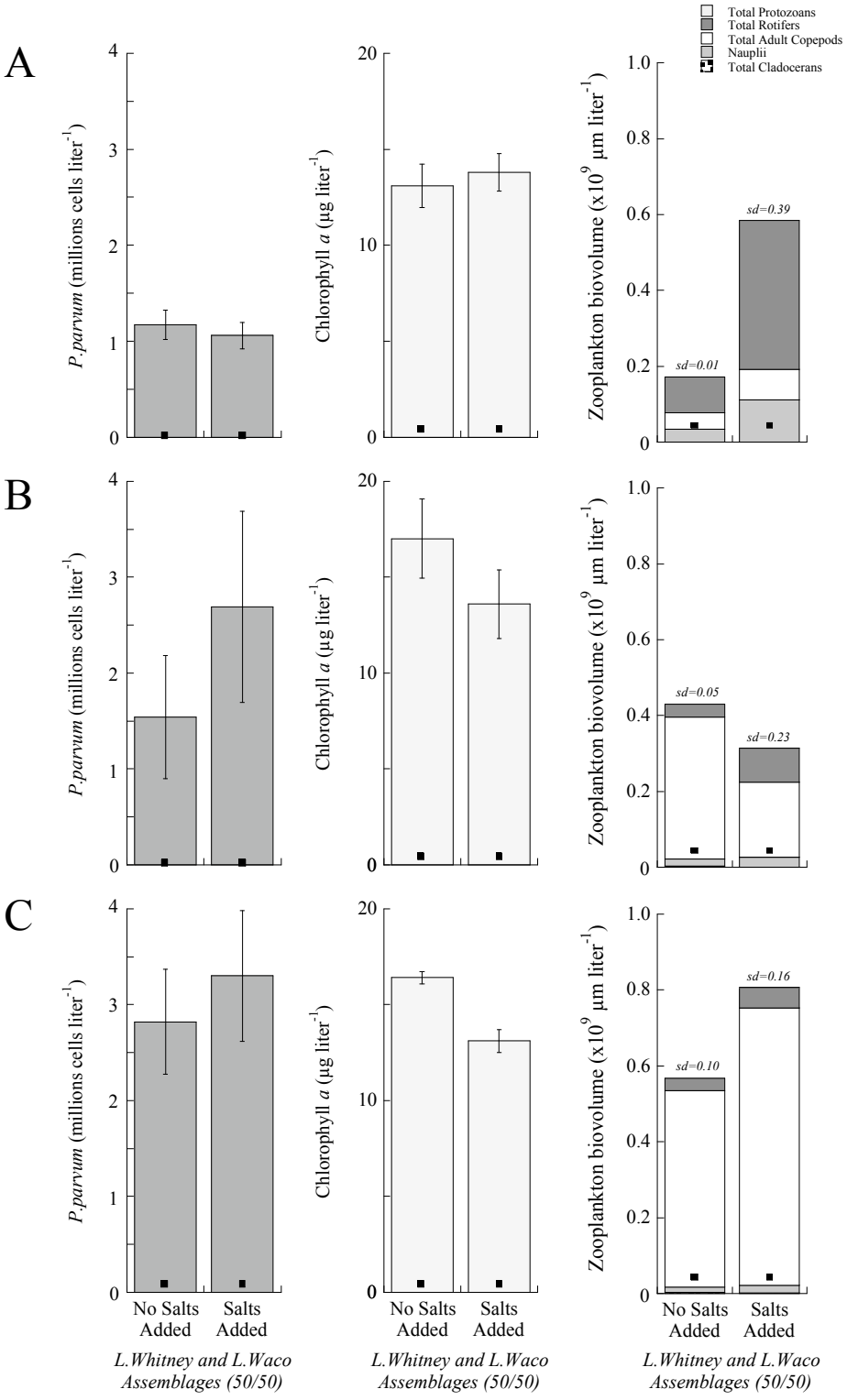
A
B
C
Total Protozoans
Total Rotifers
Total Adult Copepods
Nauplii
Total Cladocerans
P.parvum (millions cells liter-1)
Chlorophyll a (µg liter-1)
Zooplankton biovolume (x10 9 µm liter-1)
sd=0.01
sd=0.39
sd=0.05
sd=0.23
sd=0.10
sd=0.16
No Salts Added
Salts Added
L.Whitney and L.Waco Assemblages (50/50)

Fig. 5

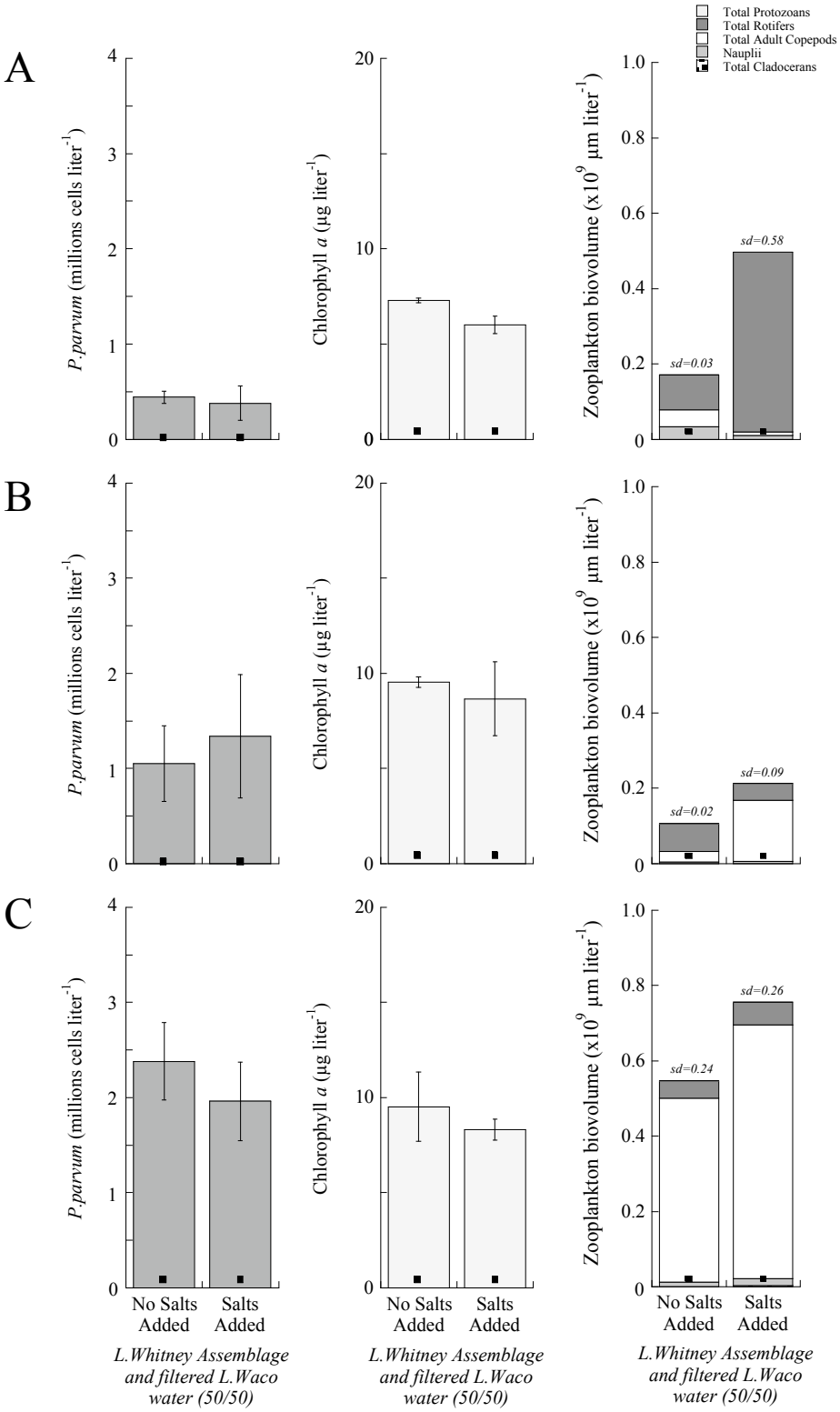
A
B
C
Total Protozoans
Total Rotifers
Total Adult Copepods
Nauplii
Total Cladocerans
P.parvum (millions cells liter-1)
Chlorophyll a (µg liter-1)
Zooplankton biovolume (x10^9 µm liter-1)
sd=0.03
sd=0.58
sd=0.02
sd=0.09
sd=0.24
sd=0.26
No Salts Added
Salts Added
L.Whitney Assemblage and filtered L.Waco water (50/50)

Fig. 6

Table 1. Contributions of cyanobacteria and Prymnesiophytes as percentages of total phytoplankton biovolume at sampling locations in Lakes Whitney and Waco at the start of each experiment.

	Experiment 1 initiated 10-3-06		Experiment 2 initiated 10-17-06		Experiment 3 initiated 10-31-06	
Taxa	L.Whitney	L.Waco	L.Whitney	L.Waco	L.Whitney	L.Waco
Cyanobacteria	49.5	77.4	18.2	68.7	28.2	51.1
Anabaena sp.	-	18.6	-	-	-	2.4
Calothrix sp.	-	-	-	-	-	5.7
Lyngbya sp.	-	-	-	1.0	-	-
Merismopedia elegans	1.9	2.1	3.8	1.2	1.0	0.8
Merismopedia punctata	0.5	1.5	-	-	-	1.2
Microcystis aeruginosa	-	36.4	-	57.3	8.8	32.9
Phormidium sp.	44.3	7.3	12.9	4.7	15.6	7.3
Raphidiopsis sp.	1.6	2.0	1.0	3.6	1.7	0.9
Synecoccocus sp.	1.2	0.7	0.6	1.0	1.0	-
unknown filament	-	8.7	-	-	-	-
Prymnesiophytes	9.6	1.7	2.8	3.0	6.2	3.5
Other Phytoplankton	40.9	20.9	79.0	28.3	65.6	45.4

Table 2. Average percent change from the initial to the final taxonomic densities (n=3) as measured using CHEMTAX. In the two columns for each experiment results are shown for the treatments where either 50% filtered water from Lake Whitney or 50% filtered water from Lake Waco was added to unfiltered Lake Whitney water.

	Experiment 1		Experiment 2		Experiment 3	
	unfiltered L.Whitney		unfiltered L.Whitney		unfiltered L.Whitney	
Taxa	+ filtered L.Whitney	+ filtered L.Waco	+ filtered L.Whitney	+ filtered L.Waco	+ filtered L.Whitney	+ filtered L.Waco
Cyanobacteria	29.6	-47*	-14.4	-58.7*	100	-38.2*
Chlorophytes	12.7	15.0	2.7	-9.2	90.6	-4.4*
Euglenophytes	-47.8	-96.8*	11.8	-100*	78.6	-99.5*
Chrysophytes	81.3	65.3	-40.8	-9.7	7.6	26.4
Diatoms	-	-	-	-	-	-
Cryptophytes	-0.2	-53.6*	-19.1	-66.9*	84.4	-30.6*
Prymnesiophytes	57.6	58.1	104	37.7*	221	107*

* significant difference ($p < 0.05$) between treatments.